Measurement for Rice Leaves Morphological Formation and Structural Information Using a Non-invasive Tomography

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***Abstract*—Using non-invasive tomography demonstrated against biological system structures involved in rolling the leaves critical of rice leaves. Rice is one of the most important crops in the world. However, rice yields are now decreasing due to unexpected changes in climate and the occurrence of mutations. Many research groups have studied this situation and important biological system structures such as bulliform cells, aerenchyma cells, and vascular bundle are involved in the rolling of rice leaves. We have noninvasive identified the structures of these rice leaves using a Swept Source Optical Coherence Tomography (SS-OCT). Furthermore, the number of small veins involved in the rolling of the leaves and the three angles based on the mid-vein quantified through acquiring tomography images. In this paper, we propose the possibility of optical coherence tomography as a biological diagnostic method for future agriculture.**

***Index Terms*—Swept Source Optical Coherence Tomography (SS-OCT), rice leaf, biological tomography imaging, morphological, structure of biological system**

I. Introduction

Recently, various studies on crops in the field of agriculture are continuing. Among these crops, rice considered the most important crop in the world [1]. Many people in the world are also seeing rice as a means of livelihood. The importance of this rice is related to the importance of the rice leaves we studied. Thanks to the importance of rice leaves, a variety of developments have been made and yields of rice have increased [2]. However, due to unexpected changes in climate and mutation, the yield of rice is now decreasing [3]. A number of research groups have conducted various studies on these phenomena and have concluded that as a result of mutation in rice leaves [4], leaf rolling occurs in the biological structure [5]. When the leaves are rolled, the photosynthetic rate of the leaves is lowered, which hinders the growth of rice. In the biological system structure of such rice leaves, the structure of rice leaves involved in rolling of leaves includes bulliform cells, parenchyma, aerenchyma, vascular bundle is a commonly known vaginal passage that is a pathway of water and nutrients. Bulliform cells are an activated cell located between the veins of rice leaves and is an important cell that regulates the rolling phenomenon of the leaves and maintains the structure of the leaves [6]. The parenchyma is a thin cell wall that serves as a constituent of the chloroplast, and this structure allows a large space between the cells, which is called aerenchyma. The aerenchyma maintains a vacuum condition by acting as a vent to exchange the gas of leaves and other tissues [7]. These structures are common structures in aquatic plants that grow semi-immersed in water, such as rice. In addition to the main structures described above, there are superficial epithelial cells, mid vein and small vein, and collenchyma which acts as a structural support for the leaves. Currently, the most representative methods for identifying and studying these biological system structures are polymerase chain reaction and microscope. The polymerase chain reaction is the most commonly used molecular technology in agriculture by analyzing DNA [8]. However, this method is disadvantageous in that the RNA is frequently contaminated during the analysis and many errors of the contaminated RNA occur. The microscope is mainly used for histological analysis such as observation of cell and tissue areas [9]. This is also a disadvantage of destroying the sample and staining with chemicals such as formaldehyde, so that the tissue is very easily damaged. The optical profile method has been proposed as an alternative to these two representative methods. The optical profile method has the advantage of not destroying the sample, and is a way to obtain a 3D view [10]. However, this technique is limited to the surface only, lacks resolution and lacks information about grasping cross-sectional images. The technology we chose to study rice leaves in our group is a non-invasive solution to the disadvantages of the three technologies mentioned above, using optical coherence tomography, a technology that allows real-time verification of three-dimensional images at high resolution respectively. Research using OCT has been conducted in various ways, and researches that have been used in various fields such as development of OCT technology [11], entomology [12] and dentistry [13]-[15] have been published. There is also

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an example of recent use in agriculture [16]-[18]. In this study, SS-OCT based on low coherence interferometry was used to acquire non-destructive tomographic images of the biological structure of rice leaves. Furthermore, the number of small veins involved in the rolling of the leaves and the three angles based on the mid-vein were quantified through acquiring tomographic images. Furthermore, the number of small veins involved in the rolling of the leaves and the three angles based on the mid-vein were quantified through acquiring tomographic images.

II. Materials and Method

A. Low Coherence Interferometry-Based SS-OCT System

The SS-OCT based low coherence interferometer was used to obtain tomography images of the biological system structure of rice leaves. System used MEMS-VCSEL sweep source lasers. Fig. 1 shows the system configuration of the utilized SS-OCT system. The OCT system is the center wavelength of 1300 nm ± 15 nm and operates at an average power of 20 mW over a broadband tuning range of 97 nm or more. The axial resolution of the system is 12 μm (in tissue) 16 μm (atmospheric) and the lateral resolution including the objective lens is 33 μm (in air) 25 μm in focus.

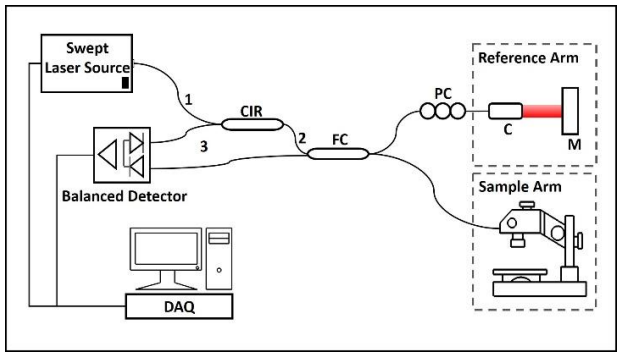

Figure 1. The schematic of the SS-OCT. C: collimator, CIR: circulator, FC: fiber coupler, M: mirror, PC: polarization controller.

B. Preparation of Sample

The CNDH population, which is most commonly used for DNA or morphological studies, was used for the study. All samples were received from the College of Agriculture and Life Sciences at Kyungpook National University. Sampling was done 40 days after sowing and all samples were rapidly cooled down to -70 ℃, where DNA and tissue were permanently stored for an extended period of time. In general, low-temperature storage is accompanied by freezing in the cell gap, resulting in rapid cell cooling. In the case of rapid cooling, the cell is cooled while maintaining its shape before the humidity changes, so when stored at room temperature, it returns to its original shape. In the process of the experiment afterwards, the image was obtained by immersing the rice leaves in clean distilled water in consideration of the leaves of aquatic plants. Images were collected within a minimum of time after removal from water to maintain freshness. The sample was randomly selected from healthy leaves and selected 6 leaves. The 6 randomly selected leaves are shown in Fig. 2. Fig. 2(a) shows photographs of the front and back of each of the 6 leaves. Fig. 2(b) shows the tomography images according to the scanning direction of the leaves. The tomography image in the red box is the image vertical to the stem direction of the rice leaf. Similarly, the tomography image in the blue box is the image horizontal to the stem direction of the rice leaves.

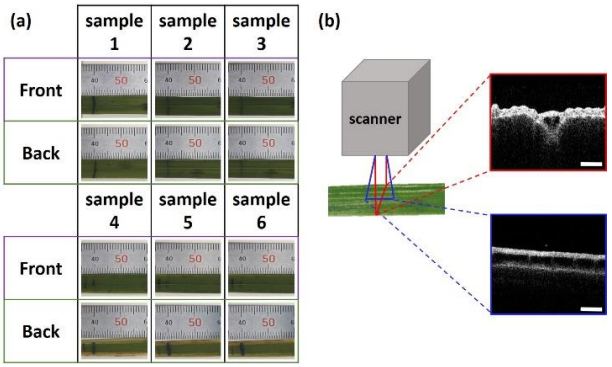

Figure 2. (a) Photographs for leaf sample. (b) is horizontal and vertical scanning direction and corresponding B-scan images. Scale bar is 500 μm.

III. Reculs and Discussion

A. Bi-directional Investigation and En-face Visualization of Rice Leaf Sample

It was analyzed by obtaining a non-invasive image with respect to the 6 rice leaves sample selected randomly. Fig. 3 shows an image of a tomographic image of a direction vertical to the direction of the stem. Fig. 3(a) and (b) are photography of the front and back of the rice leaves surface. The dotted red arrows in Fig. 3(a) and (b) show the scan direction and ROI area for Fig. 3(c) and (d). The scan direction is the same as the red triangle in Fig. 3(c) and (d). In Fig. 3(c) and (d), the upper and lower epidermis cells, the bulliform cells involved in the rolling of leaves, and the aerenchyma acting as vents were clearly observed. In addition, it shows that the important structures involved in the growth of rice leaves such as mid-vein, small vein, and vascular bundle were distinctly identified non-invasively.

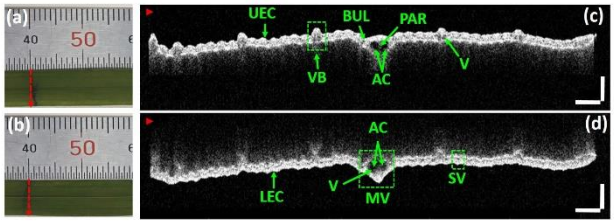

Figure 3. Compares two-dimensional SS-OCT images of the front and rear surface of the rice leaf sample. (a) and (b) show the front and back surface of the rice leaf sample. (c) and (d) are tomography images of the dotted red arrows in a and b. AC: Aerenchyma, BUL: Bulliform cells, LEC: lower epidermis cells, MV: mid-vein, PAR: parenchyma, SV: small vein, UEC: upper epidermis cells, V: vacuum region and VB: vascular bundle. Scale bar is 500 μm.

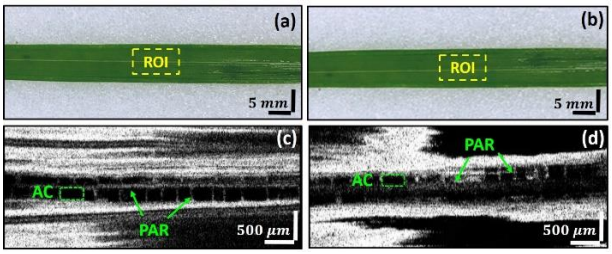

Figure 4. (a) and (b) are real photographs of rice leaves, (a) is front and (b) is back. (c) and (d) are en-face images of the yellow ROI regions shown in (a) and (b). Region of the ROI is based on mid-vein. AC: Aerenchyma, PAR: parenchyma.

Fig. 4 shows an en-face image based on the mid-vein. Fig. 4 (a) and (b) show the front and back of a rice leaf. Fig. 4(c) and (d) show the en-face images of the yellow box ROI regions express in Fig. 4(a) and (b). In the images of Fig. 4 (c) and (d), parenchyma and parenchyma forming a thin cell wall form a large space. This is called aerenchyma, which exchanges gas with other tissues in the vacuum space. The important structure could be seen in Fig. 3, was confirmed to be the same in different scan directions. Thus, using a non-invasive tomography technology demonstrated that it is possible to confirm the important structure of the rice leaves through a variety of scanning directions. In addition to the tomography images, the structure of the rice leaf surface was also confirmed. Fig. 5 shows that the surface of rice leaves can be observed through three-dimensional images. Fig. 5(b) and (e) are three-dimensional images of the front and back of the rice leaf surface. Fig. 5(a) and (c) are enlarged images of the area shown in Fig. 5(b). Similarly, Fig. 5(d) and (f) are magnified images of the area shown in Fig. 5(e). In Fig. 5(a), the surface pattern of rice leaves and vascular bundle, which is a movement path between water and nutrients, can be seen through three-dimensional images. In addition, the red arrowheads shown in Fig. 5(c) and (f) represent projections observed on the surface of the enlarged rice leaf sample.

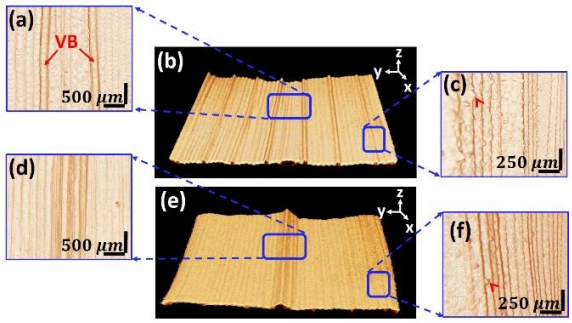

Figure 5. Three-dimensional image acquired using SS-OCT. (b) shows the front side of the rice leaf surface, and (e) shows the back side of the rice leaf surface. (a) and (c) are enlarged images of the blue square boxes shown in (b). (d) and (f) are enlarged images of the blue square box of (e). VB: vascular bundle.

B. Quantitative Measurement of Morphological Characteristics

We measured the following information about rice leaves. The number of small veins on rice leaves, three angles based on mid-vein, and the layer thickness of leaves were measured. Fig. 6 shows the measurement method. Fig. 6(a) shows how to measure the number of small veins. Fig. 6(b) measures the three angles relative to the mid-vein, and these three angles can be used to quantitatively identify the rolling behavior of leaves when mutations occur. Finally, the thickness of the leaf layer was measured in the same manner as in Fig. 6(c).

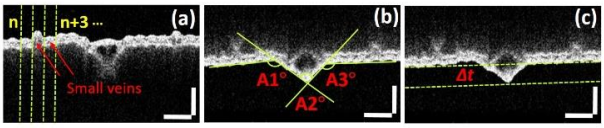

Figure 6. Number of small veins, three angles based on mid vein A1~3 and leaf layer thickness measurement procedure. Scale bar is 500 μm.

The measured values in Fig. 6 are summarized in Table I. The average number of small veins is about 40. The layer thickness of the leaves is to measure the size of the mid-vein. As for the three angles, the angles of A1 and A3 are generally larger than the values of A2. The difference in the measurements of the leaf layer thickness is large, but shows an average of about 260 μm.

TABLE I. NUMERICALLY ENUMERATED AVERAGE NUMBER OF SMALL VEINS, ANGULAR INFORMATION AND THICKNESS OF HEALTHY SAMPLE

	Sample 1	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**	**Sample 6**
Small vein [ea]	41	45	42	41	41	35
A1 [°]	122.6	135.93	137.29	144.33	126.18	163.88
A2 [°]	94.18	94.01	100.06	98.04	102.08	121.18
A3 [°]	134.33	132.37	136.18	131.48	152	144.95
Δt [μm]	283.34	569.46	294.537	253.34	78.21	126.12

IV. CONCULSION

In this study, cross-sectional photographs of the biological system structure of rice leaves were obtained non-invasively using SS-OCT based on low interferometers. The acquired tomography images clearly identify the important structures of rice leaves that can be identified by standard methods. In addition, the measurement was carried out to numerically confirm the leaf rolling phenomenon when mutation occurs. Based on these results, we expect to be able to distinguish between mutant leaves and healthy leaves. To date, there are no studies on rice leaves using SS-OCT. This technique is presented as a way to identify the biological system structure of rice leaves. SS-OCT can also be identified as a potential new technology in agriculture.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Hyeree Kim analyzed the data and wrote the overall paper; Ruchire Eranga Wijesinghe conducted data

analysis and reviewed and edited papers; Mansik Jeon and Jeehyun Kim conceptualized and verified the paper; all authors had approved the final version.

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